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Evaluating the Potential of Adipose Tissue-Derived MSCs as Anticancer Gene Delivery Vehicles to Bone-Metastasized Prostate Cancer

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14. ABSTRACT(approximately 200 words)
The purpose of this project is to demonstrate that adipose tissue derived MSCs (AT-MSCs) have tumor-homing potential and can be used to deliver anti-cancer genes to bone metastasized prostate cancer (PCa) cells. The ease of isolation, culturing and genetic transduction of AT-MSCs make them attractive candidates for gene-therapy approaches against cancers. The purpose of our proposed studies is to enrich for those AT-MSCs which have better tumor-homing potential and demonstrate that these enriched AT-MSCs carrying a suicide gene (HSV-TK) will localize to PCa tumor foci within the bone and chemosensitize the cancer cells to ganciclovir (GCV). We have obtained several stocks of primary AT-MSCs and characterized them for their mesenchymal stem cell lineage. We have succeeded in stably transducing them with a fluorescent reporter (GFP). In trans-well *in vitro* studies, we have isolated cells with enhanced tumor-homing potential and have been able to increase their invasion and migration towards tumor-derived factors. We have enriched these subpopulations and have initiated their GFP transduction. By gene-array analysis, we have identified several surface markers that may enable the invasive phenotype in AT-MSCs. These markers would enable us to enrich for AT-MSCs as gene delivery vehicles and our approach using HSV-TK/GCV would facilitate the development of an optimal anti-cancer strategy to eliminate the bone PCa foci in a tumor-xenograft model *in vivo*.

15. SUBJECT TERMS
Prostate Cancer, Adipose Stem Cells, Characterization, Lentiviral transduction, in vitro Tumor-homing, Enrichment, Surface markers, Differentiation potential

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Annual Report (PC080811): 04/01/2009 – 03/31/2010

Introduction:

The ease of isolation, culturing and genetic transduction of AT-MSCs make them attractive candidates for cellular and gene-therapy approaches. The overall goal for our project is to demonstrate that, similar to bone marrow derived MSCs (BM-MSCs) adipose tissue derived MSCs (AT-MSCs) also have tumor-homing potential and can be used to deliver anti-cancer genes to bone metastasized prostate cancer (PCa) cells. Indeed, several publications in 2009 and 2010 have shown clear evidence of tumor-site specific localization of AT-MSCs [1-3]. However, these studies have only investigated the AT-MSC recruitment to sub-cutaneous tumors in nude mice, but their ability to traverse the vasculature and colocalize with intraosseous PCa tumor foci, have not been demonstrated. Furthermore, these studies have documented that only a low percentage of the systemically injected AT-MSCs are actually recruited to the tumors. The innovative aspect of our proposed studies is that we plan to enrich for those AT-MSCs which have better tumor-homing potential. We have optimized several *in vitro* strategies for isolation of cells with enhanced invasion and migration towards tumor-derived factors. We have obtained several different stocks of primary AT-MSCs from male lipoaspirates, characterized each line for their stem cell marker and lineage specific differentiation properties. In the enriched subpopulations that have tumor predilection, we have started the identification of surface markers that are associated with the invasive phenotype of AT-MSCs. From studies with *in vitro* enriched cells, we believe that approaches that can activate the invasive phenotype and sort for those AT-MSCs with significant tumor-homing abilities, would facilitate the development of an optimal anti-cancer gene delivery vehicle to PCa foci *in vivo*. During the first year of our DOD-funded studies we have been able to address most of the experiments outlined in *Task-1*. Within the 'Body', we have listed our findings according to each of the 'Sub-Tasks'. The salient findings and important observations generated from these initial investigations, are also provided in the 'Key research accomplishments' section.

Body:

Task-1. *In vitro* evidence of AT-MSC recruitment to PCa foci and enrichment of those AT-MSCs possessing enhanced tumor-tropism.

1.1. *Isolation and culturing of AT-MSCs from lipoaspirates.*

1.2. *Analysis of stem cell specific markers and characteristics of AT-MSCs.*

In contrast to female donors, the availability of male donors for lipoaspirates has been difficult, however, with Dr. Izadpanah's and Dr. Gimble's assistance, we have generated eight AT-MSC stocks for our studies. According to our previous published protocols [4, 5] we have isolated abdomen lipoaspirates from caucasian males, ages 25-60 yrs and body mass index (BMI) of 26.56 to 33.80. Briefly, lipoaspirates (300 ml) were washed thoroughly (4-5 times) in phosphate buffered saline (PBS) with antibiotics, and subjected to enzymatic digestion using 0.2% Collagenase-A type I (Sigma-Aldrich) at 37^{o}C for 2 hrs. The single-cell suspension is centrifuged using a Histopaque gradient and cell pellets were resuspended in complete medium (DMEM:F12 medium containing 50% FBS, antibiotics and fungizone) and subjected to serial filtration through 100 μm and then 40 μm nylon filters. The AT-MSCs adhere to plastic surfaces within 24 hours after which the medium was changed and cells were allowed to propagate. This protocol for AT-MSC culturing and propagation enabled generation of large quantities of each of these stocks which have been cryopreserved in liquid nitrogen, for use in our long-term studies towards this project. In four AT-MSC stocks, we have characterized the stem cell surface markers and monitored their differentiation potential towards adipocytes, chondrocytes or osteoblasts (Table-1).

Table 1: Characterization of AT-MSCs

Surface Positive Markers	***Surface Negative Markers***	***Differentiation Potential***
CD44, CD49b, CD49c, CD29, CD105, CD146, CD14, CD13, CD54, CD55, CD59, CD71, CD73, CD90, CD106, etc.	CD11, CD14, CD16, CD18, CD31, CD45, CD50, CD56, CD62, CD104, Factor VIII related Ag, HLA-DR	Adipocyte Chondrocyte Osteoblast

Surface markers were analyzed by flow cytometry using fluorescent conjugated antibodies. A representative flow-data is shown in Fig.1. and a representative differentiation specific staining is shown in Fig. 2.

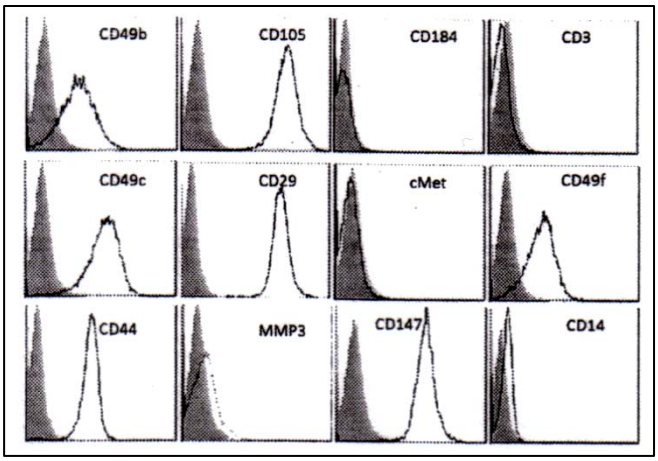


Figure-1. A representative (AT-MSC #07) flowcytometry data of AT-MSC surface markers are shown. Cells ($2X10^5$) were incubated with FITC or PE-conjugated antibodies for 30 mins at RT, washed and percent fluorescence positive cells were measured using a FACScan flowcytometer. Nonspecific fluorescence was determined using cells incubated with anti-mouse IgG (filled peak).

We have also compared the adipogenic, osteogenic and chondrogenic differentiation potential in each of these AT-MSC stocks. A representative (#04) is shown along with comparative data obtained in bone marrow derived MSCs (BM-MSCs) line (Fig.2;A-H).

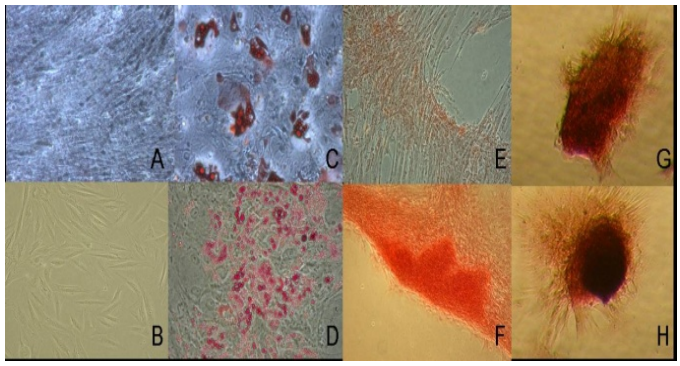


Figure-2. Morphology, proliferation and differentiation potential of adult MSCs. Culture expanded AT-MSCs (A) and BM-MSCs (B) demonstrate the spindle shaped fibroblastic morphology. BMSCs and AT-MSCs retain multilineage differentiation capabilities, undergoing adipogenesis (C and D), osteogenesis (E and F), and chondrogenesis (G and H) *in vitro*.

The specifics on these four well characterized AT-MSC batches are given below:-

Number	P assage	CD29	CD105	CD45	CD34	CD44	CD73	CD90	PE Ctrl	FITC Ctrl	PBS Ctrl
AT-MSC-01	P-0	98.6	97.10	4.3	89.25	5.25	93.55	85.65	3.59	1.34	2.1

Race: Caucasian Gender: Male **Age: 34** Height: 5 ft. 7 inches

Weight: 196 lb **BMI= 27.25** Depot: Abdomen Lipoaspirate

Number	Passage	CD29	CD105	CD45	CD34	CD44	CD73	CD 90	PE Ctrl	FITC Ctrl	PBS Ctrl
AT-MSC-03	P-0	99.5	99.15	4.21	84.32	12.26	58.1	89.9	2.65	2.48	2.14

Race: Caucasian Gender: Male **Age: 18** Height: 5 ft. 10 inches

Weight: 185 lb **BMI= 26.56** Depot: Abdomen Lipoaspirate

Number	P assage	CD29	CD105	CD45	CD34	CD44	CD73	CD90	PE Ctrl	FITC Ctrl	PBS Ctrl
AT-MSC-04	P-0	99.23	98.41	4.39	89.51	5.12	93.45	85.45	2.57	1.56	1.89

Race: Caucasian Gender: Male **Age: 46** Height: 5 ft. 9 inches

Weight: 212 lb **BMI= 31.8** Depot: Abdomen Lipoaspirate

Number	P assage	CD29	CD105	CD45	CD34	CD44	CD73	CD90	PE Ctrl	FITC Ctrl	PBS Ctrl
AT-MSC-07	P-0	99.56	99.61	4.9	88.55	5.42	90.85	83.05	2.53	1.23	1.9

Race: Caucasian Gender: Male **Age: 55** Height: 5 ft. 8 inches

Weight: 222 lb **BMI= 33.8** Depot: Abdomen Lipoaspirate

Results: We have isolated several AT-MSC lines from lipoaspirates and their characterization has been completed. From 300 ml of lipoaspirates we routinely isolated up to 10^7 adipose stem cells with greater than 95% purity. However, yields can vary widely between donors, and differences in both proliferation and differentiation potentials were also observed.

Using these four batches of AT-MSCs, we have carried out invasion and migration assays (presented later).

1.3. *Lentivirus transduction of AT-MSCs with LV269 and analysis of GFP-fluorescence*.

Lentiviral (LV) transduction of stem cells is a challenge and efficiency tends to be low. Using published protocols for MSC transduction by our collaborators, Drs. Bunnell and Reiser, we have been able to optimize the protocol in AT-MSCs, by using polyethylenimine from transfection efficiency and generation of a LV packaged in vesicular stomatitis virus glycoprotein (VSVg) [6, 7]. From the local 'Vector Core' facilities, we have obtained high titers (MOI $>10^8$/ml) of this LV which express green fluorescent protein (GFP) under the control of cytomegalovirus (CMV) promoter. Briefly, the LV was generated by using three-plasmids [550 μg of each of the lentivirus plasmid (pCMV-GFP); GAG and POL expression plasmid (pCMV-R8.74) and VSVg expression plasmid (pMD2.G)] which were transfected into 293T cells. Virus was concentrated by ultracentrifugation at 25,000 rpm for 90 minutes at 4°C. Viral concentrates were titered against 293T cells to establish the multiplicity of infection (MOI). For all experiments, AT-MSCs were transduced within the first three passages following initial plating and at less than 30-40% confluence. Cells were exposed to 10-50 MOI of the virus for 16 hours, thoroughly rinsed with PBS and returned to basal media. At 48 hours following transduction, cells were analyzed by flow cytometry (Fig. 3a & 3b) and immunofluorescence microscopy for transduction efficiency (Fig. 3c). Cells were cultured for 1-week and the high GFP-expressors were sorted by FACSorting (Fig. 3d). These cells maintained high level of GFP-fluorescence for at least 4-6 weeks in culture. Cryogenic stocks of these cells have been generated.

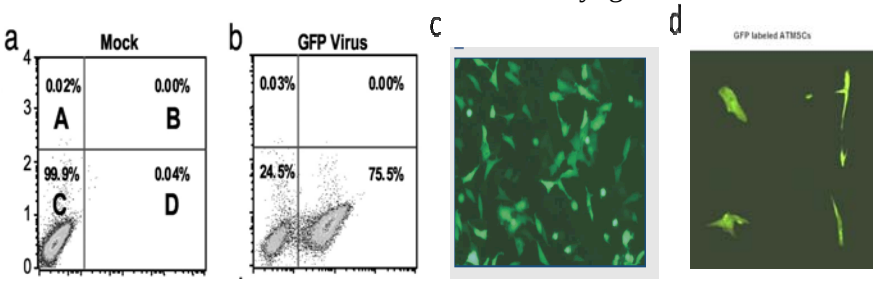


Figure-3: The GFP specific flow-cytometry (a & b) and fluorescence microscopy (c) data of a representative transduction in AT-MSC (#07) is shown. Cells that retained GFP-fluoresence after 1-week were FACSorted for high GFP expressors (d) for propagation and freezing.

Results: By using polyethylenimine-mediated transfection protocols to generate high lentivirus titre and by using LV expressing the VSV-Glycoprotein [7], we have been able to demonstrate that AT-MSCs can be transduced

efficiently (>40-50 %) at least in the short term. High efficiency of lentivirus transduction with LV-GFP was achieved in almost all (four) AT-MSC stocks. A lower amount of cells stably expressed the reporter gene (GFP) for at least one month following transduction. The cryogenically stored transduced cells also showed stable expression of GFP following their thawing and reculturing.

1.4. *Set up the transmigration experiments in trans-well culture (TWC) chambers.*

We have used four different AT-MSC batches (01, 03, 04 and 07) to monitor their *in vitro* migration and invasion towards either serum free medium (S.F.M.), medium containing 10% FBS or towards tumor-derived factors (C4-2B conditioned medium) to specifically determine tumor-homing ability of AT-MSCs. In order to identify those cells which can efficiently traverse membrane barriers and those which actively invade through extracellular matrix (ECM), both migration (Fig. 4A) and invasion assays (Fig. 4B), were carried out. In the migration assays, we measured percentage of cells travelling via the transwell membrane insert only (8 μ pore size) after 6 hrs. For the invasion assays, percentage of cells travelling through a matrigel barrier followed by migration via transwell membrane insert, were determined at 16 hrs. These two different assays were carried out to critically demonstrate that certain AT-MSCs have active migration and invasion ability towards tumor-derived factors, which requires the actions of cell adhesion molecules (CAMs), integrins and matrix metallo-proteases (MMPs), rather than simple migration towards a concentration gradient of growth factors and/or chemokines.

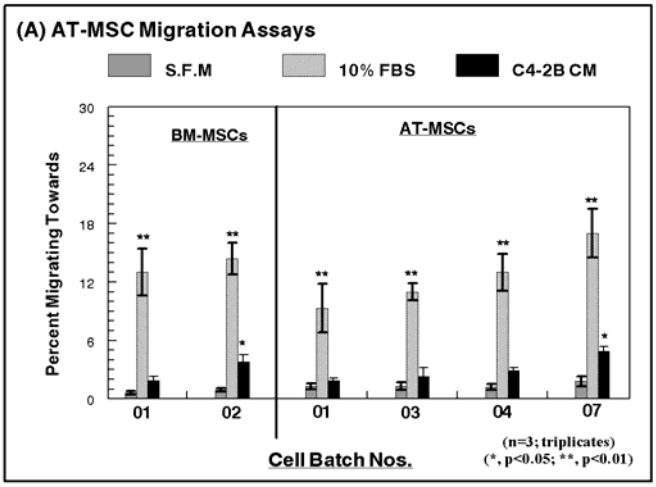


Fig. 4A

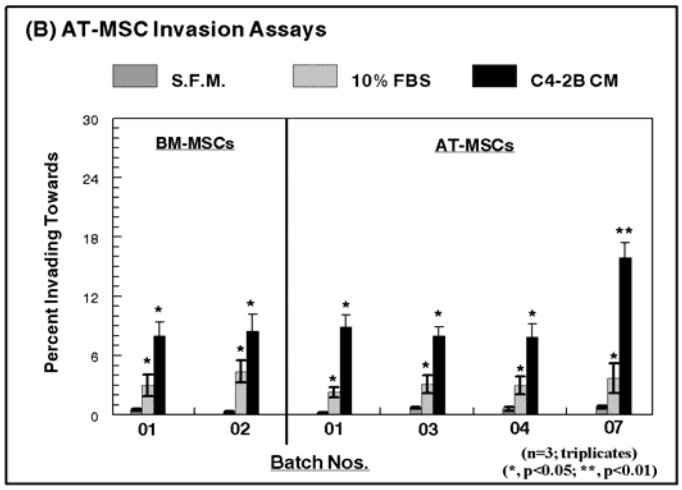


Fig. 4B

Figure-4: Differential attraction of BM-MSCs and AT-MSCs towards tumor-derived factors. For these studies, calcein-AM labeled cells were added to the upper chamber of a 12-well transwell chamber and S.F.M, FBS or C4-2B CM was added to the bottom chamber. Percent of cells actively travelling to the lower chamber was determined by fluorescence determination in the top and bottom chambers by using an Flx800 fluorimeter.

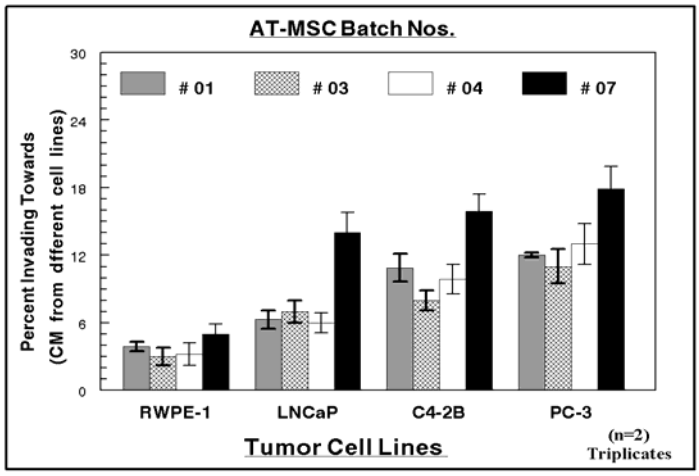


Fig. 5

Next, we wanted to observe whether different PCa tumors release factors which differentially attract AT-MSCs. Cells invading towards CM obtained from either less aggressive (RWPE-1 and LNCaP) or more aggressive PCa cell lines (C4-2B and PC-3) were used for these studies.

Figure-5: Effect of different CMs from different PCa lines on AT-MSC invasion. The invasion assays were carried out similar to the previous experiments to demonstrate the crucial role of factors released from highly aggressive tumors, and shows that certain AT-MSCs have better tumor-homing potential.

We have carried out further studies in AT-MSCs in order to determine whether *in vitro* manipulation of AT-MSCs can be carried out to enhance their migratory and invasive abilities towards tumor cells (Fig. 6). The trans-well culture studies were carried out using AT-MSCs which were stimulated for 2 hrs with either TNF-α (10 U/ml), IL-1β (10 U/ml) or TGF-β1 (2 U/ml), following which they were washed and then cultured for 16 hrs before labeling with Calcein-AM and use in trans-well invasion assays. In addition, cells grown under serum deprived (SD) condition for 2-3 days, were also used to determine growth factor or tumor-derived factor specific migrations.

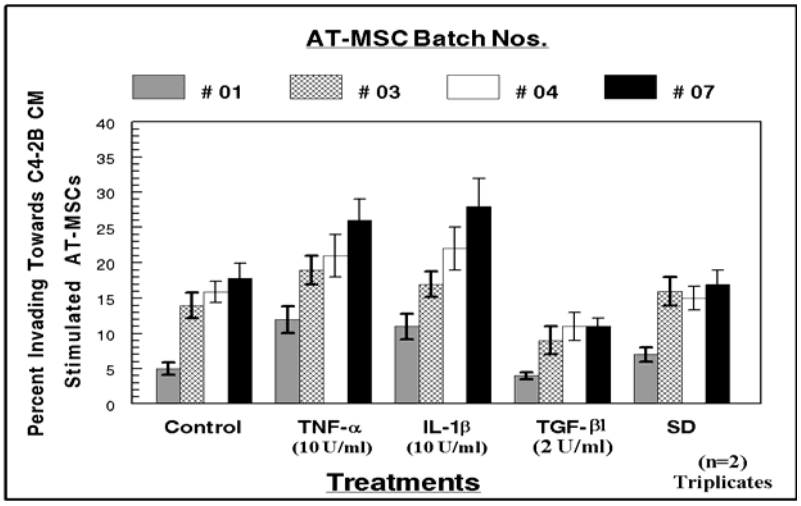


Fig. 6

Figure-6: Effect of different stimulations on AT-MSC invasive ability towards C4-2B conditioned medium. For the *in vitro* AT-MSC manipulation studies, cells from four different AT-MSC batches were exposed to cytokine stimulation or serum deprivation. The migrations of unstimulated (control) or stimulated AT-MSCs towards C4-2B CM were analyzed at 16 hrs and percent of cells in the lower vs upper chambers of the transwell plates were calculated by using an Flx800 fluorimeter.

Results: Our findings clearly showed that AT-MSCs have tumor homing properties *in vitro* which were comparable to that observed with BM-MSCs. Specific migrations towards tumor-derived CM were only observed in the matrigel invasion assays and not in their migration towards 10% FBS. In the absence of matrigel, more cells migrated towards 10% FBS and less towards tumor-CM. However, in the invasion assays, higher percentage of cells were able to traverse the matrigel layer when tumor-CM was placed in the bottom chamber. We further observed that tumor-tropism of AT-MSCs was especially higher towards the aggressive prostate tumor cell lines, e.g. C4-2B and PC-3, as compared to either LNCaP or RWPE-1 cell derived CM. Prior activation of these cells with TNF-α or IL-1β, but not TGF-β1, increased migration towards tumor-derived CM, however, growth factor deprivation (SD-cells) only showed higher migration towards 10% FBS. Interestingly, we observed a trend towards higher level of invasion in AT-MSCs from donors with higher BMI (#04 & #07) as compared to those with lower BMI (#01 & #03), possibly indicating an inherently activated phenotype of AT-MSCs in obese individuals.

The above studies have enabled us to enrich for several AT-MSC clones with high migratory potential. We have initiated studies on molecular marker identification on these clones which we plan to utilize in our *in vivo* tumor-homing studies, as specified in our later Tasks.

1.5. *Immunohistochemistry (IHC) to identify AT-MSCs colocalizing with the PCa foci.*

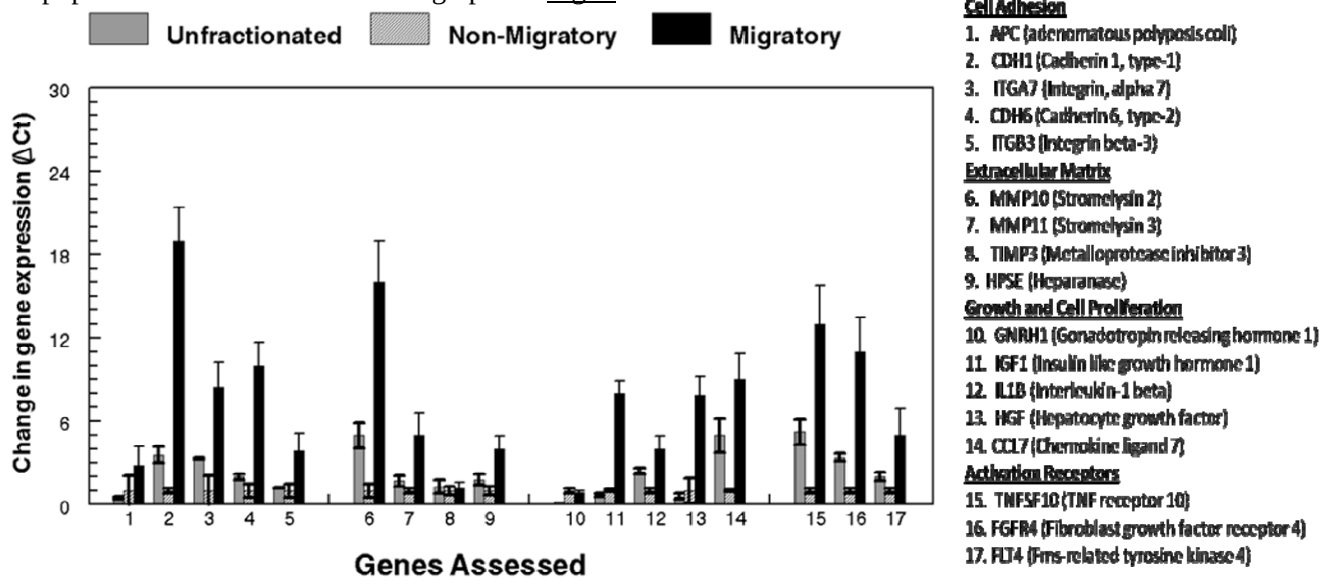
Our initial migration and invasion studies showed promising data towards possible enrichment of tumor-tropic AT-MSC by using tumor derived CM only and we were successful in identifying and propagating specific clones. Hence, the coculture studies with PCa cells to further show colocalization of AT-MSCs, have not been initiated. However, we have focused our attention towards characterization of the AT-MSC clones that were isolated and enriched from the initial migration and invasion assays.

Our ultimate goal will be to demonstrate that FACSorted subpopulation of AT-MSCs possess a much better ability of to seek-out the tumors at metastatic sites, both *in vitro* and *in vivo*. We have generated significant preliminary data on their surface phenotype profile (*Task-1.6. & Task-2.1.*). Once we establish tumor-site specific homing protocols, we plan to use these enriched cells, as well as cells directly FACsorted from the unfractionated AT-MSC population, and utilize them for our *in vivo* tumor-homing experiments in nude mice. This would be the proof-of-concept data for our proposed hypothesis and needs to be carried out as soon as possible.

The animal use protocol has been approved by the Tulane IACUC committee and we have initiated experiments in the nude mice containing sub-cutaneous PCa tumors. Using the GFP-labeled AT-MSCs, we can measure AT-MSC colocalization with the tumor foci. In two well characterized stocks of cells; one obtained from an older (#07: age:55, BMI=33.8) and an younger (#03: age:18, BMI=26.56) individual, we have generated significant differences in migratory and invasive abilities and have been able to determine differential expression of surface markers via gene-array analyses.

1.6. *Sorting of AT-MSCs and isolation of RNA and protein, for further characterization of differential markers.*

From the *in vitro* studies we have isolated several clones of AT-MSCs that possess enhanced migratory ability. These cells were propagated and aliquots of cells have been used for further studies to monitor differential expression of genes. Expression of genes which are closely associated with cell adhesion and migration [8-14] were compared in the unfractionated (total), non-migratory and the migratory subpopulations of AT-MSCs. In our preliminary studies, we focused on the expression of integrins (Int), cadherins (Cdh) and matrix metallo-proteinases (MMPs). We compared the expression of markers in the migratory population and the congruent non-migrated population from the same AT-MSC batch by *Real-time* RT-PCR assays and fold changes in expression of specific genes were determined by using the RT2 Profiler custom PCR array (SuperArray Biosciences, # PAHS-028D). Briefly, in a 96-well plate SuperArray system we simultaneously examined the mRNA levels of 89 genes including five 'housekeeping genes', according to the Manufacturer's protocols. The first-strand cDNAs were synthesized from 2.5 μg of total RNA and quantitative PCRs were performed independently for each subpopulation obtained from AT-MSC (cell clone #03). Values obtained for the threshold cycle (Ct) for each gene were normalized using the average of four house-keeping genes (HPRT1, RPL13A, GAPDH, ACTB). Analyses were performed using the software provided by the manufacturer, and the fold change (ΔΔCt) between the unfractionated, non-migrated and the migrated populations were assessed. The genes which showed highest differences in expression between the subpopulations are shown in the bar graphs in Fig. 7.



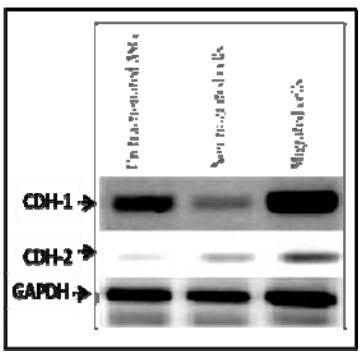


Figure-7 (above): AT-MSC subpopulations showing several differentially expressed genes, as observed using the RT2 Profiler custom PCR array. The table in the right shows the gene names.

Figure-8 (left): Semi-quantitative RT-PCR determination of Cdh-2 and Cdh-6 mRNA levels in AT-MSC subpopulations. Data with two of the highly differentially expressed genes, e.g. CDH-1 and CDH-6 were further confirmed by direct semi-quantitative RT-PCR analysis and the RT-PCR products are shown in the representative gel picture (Fig. 8).

6

Results: Several of the genes associated with 'cell adhesion', 'extracellular matrix' and 'activation receptor' expression were amongst the most upregulated genes in the migratory AT-MSCs. The migratory subpopulation expressed significantly higher levels of Cdh-1, Cdh-6, Int-α7, HGF, IGF-1, both TNF and FGF receptors, as well as both MMP-10 (stromelysin-2) and MMP-11 (stromelysin-3) [8-14].

1.7. *To monitor adipogenic and osteoblastic differentiation in AT-MSC subpopulations.*

We have carried out studies to determine the effect of tumor-derived factors on AT-MSC differentiation towards either adipogenic or osteogenic lineages. These studies on lineage-specific differentiation and the effect of tumor-derived factors, will enable us to develop a strategy to increase the localized expression of the anti-cancer genes (i.e. HSV-TK) in AT-MSCs following their recruitment at the PCa tumor foci. For adipogenic differentiation, cells were incubated in DMEM medium (0.5 μM 1-methyl-3 isobutylxanthine, 1μM dexamethasone, 10 μg/ml insulin and 100 μM indomethacin) for 3 weeks. To visualize the extent of lipid droplets, cells were fixed with 4% formalin and stained with Oil red-O dye (Fig. 9A). For osteogenic differentiation, cells were incubated in medium containing 100 nM dexamethasone, 10 mM β-glycerophosphate and 0.05 mM L-ascorbic acid-2-phosphate for 3 weeks. Mineralization of the extracellular matrix was visualized by staining with Alizarin red dye (Fig. 9A). The extent of differentiation was measured colorimetrically by organic phase extraction (isopropanol) of the dye and measuring fold change in optical density (OD). Data are presented in the bar graphs (Fig. 9B).

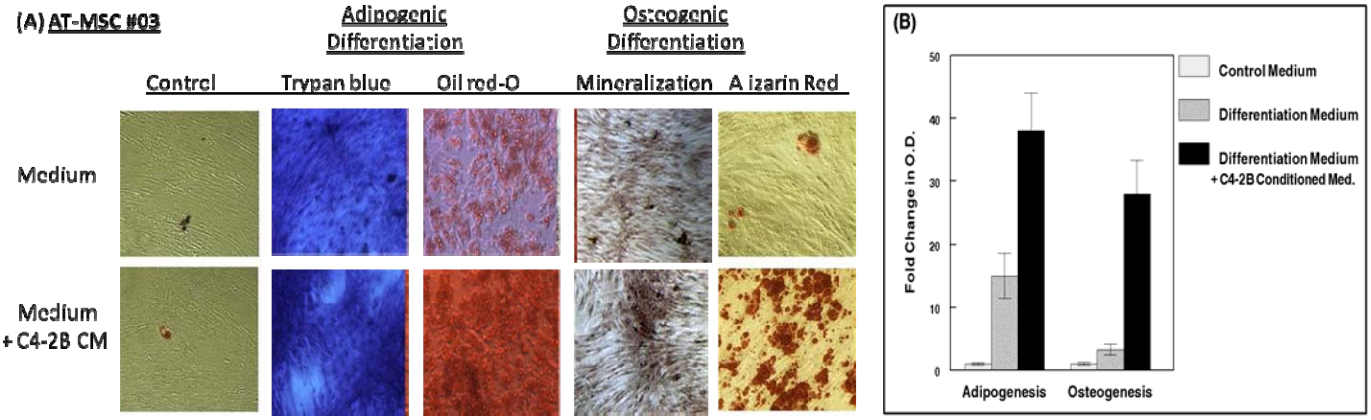


Figure-9: Lineage specific differentiation of AT-MSCs in presence of tumor-derived factors. In (A), cells stimulated with differentiation medium alone or in combination with C4-2B conditioned medium were stained for adipogenesis and osteogenesis. The stain was extracted and extent of differentiation quantified (B).

In the following studies, we have compared the extent of adipogenic differentiation (oil red-O staining) in unfractionated AT-MSC, as well as in both migrated and non-migrated subpopulations of AT-MSCs (Fig. 10).

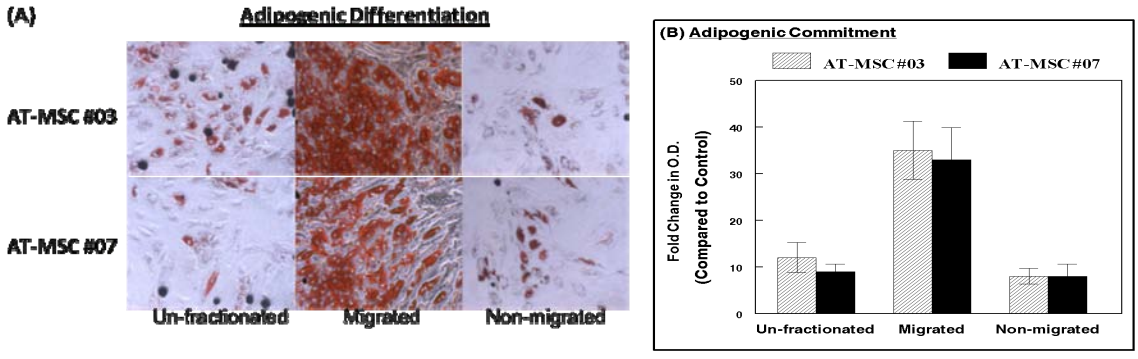


Figure-10: Adipogenic differentiation in AT-MSC subpopulations. To measure the most change in adipogenesis commitment and lipid accumulation, cells were stained after 2-weeks in these experiments (Fig. 10A). The extent of differentiation was measured colorimetrically and fold change in optical density (OD) compared to undifferentiated cells (controls) are presented in the bar graphs (Fig. 10B).

Results: We observed that in presence of differentiation medium and tumor-CM both adipogenesis and osteogenesis of AT-MSCs are significantly increased. As compared to BM-MSCs, we observed enhanced

adipogenic differentiation potential of AT-MSC (>50% oil red-O positive cells within 2 weeks) as compared to osteogenic differentiation in BM-MSCs (not shown). Furthermore, and very interestingly, as evidenced by a significantly increased oil red-O staining intensity, the migratory population of AT-MSCs differentiate better and faster towards the adipogenic pathway (>95% oil red-O stained cells within two weeks).

1.8. *In osteoblastic AT-MSCs, measurement of Osx activity and α1-Collagen (COL11A2) expression (RT-PCR) and promoter activity (Luciferase assay).*

We have recently initiated these studies in the unfractionated, non-migratory and migratory subpopulation of AT-MSCs. We are currently monitoring the increase in both the rate and extent of adipogenic and osteogenic transcription factors during early time points post stimulation (6-24 hrs). Furthermore, the gene-array and proteomic analysis of lineage committed AT-MSCs following their exposure to tumor-derived factors, is also planned [15, 16]. The above findings clearly indicated that the migratory subpopulation of AT-MSCs may be pre-committed and may accelerate towards the differentiation pathway in presence of tumor-derived factors and differentiation stimulation. Indeed, this molecular process may be taken advantage of in the genetically transduced AT-MSCs to enhance HSV-TK expression following their tumor-site specific recruitment. Data from these and other planned studies will be included in the next annual report.

Key Research Accomplishments:

- In several well characterized AT-MSC batches, our *in vitro* studies showed that only a small subpopulation of these cells are preferentially attracted towards PCa derived factors.
- Simple 'migration' and 'matrigel invasion' assays were able to demonstrate that *in vitro* enriched clones of AT-MSCs have differential attraction towards tumor-derived factors, especially from aggressive PCa cells.
- *In vitro* stimulation with either TNF-α or IL-1β increased the percentage of this migratory phenotype even in the unfractionated AT-MSCs.
- Certain AT-MSCs possess higher migration and invasion ability towards tumor derived factors, however, serum deprivation of AT-MSCs only enhanced their migration towards growth factors and not towards CM.
- In AT-MSCs enriched for their invasive abilities, significantly increased expression of several adhesion and migration associated genes, e.g. Cdh-2, Cdh-6, Int-α7, HGF, IGF-1, TNFSF10 and FGFR4, and MMP-10 and MMP-11, were observed.
- Coexposure to tumor derived factors (C4-2B CM) enhanced both adipogenic and osteogenic differentiation of AT-MSCs.
- The *in vitro* enriched clones of AT-MSCs, migratory subpopulation, showed significantly increased adipogenic differentiation.

Reportable Outcomes:

1. Poster Presentation at Tulane Health Science Research Day, March 3-4, 2010, New Orleans, LA.

ADIPOSE TISSUE DERIVED MESENCHYMAL STEM CELLS: INVASION, DIFFERENTIATION AND APPLICATION IN TARGETED DELIVERY TO BONE METASTASIZED PROSTATE CANCER. Whitney Sherman*, Rachel Gelfond, Geetika Chakravarty & Debasis Mondal. Department of Pharmacology, Tulane University Health Sciences Center, School of Medicine, New Orleans, LA-70112.

2. Poster Presentation at AACR Conference, April 17-21, 2010. Washington DC.

Nrf1 and Nrf2 mediated regulation of androgen receptor signaling in androgen-independent prostate cancer cells. Michelle Schultz*, Asim B. Abdel-Mageed, Krishna C. Agrawal, Geetika Chakravarity and Debasis Mondal. Tulane University Medical Center, New Orleans, LA-70112.

Conclusions:

We have demonstrated that similar to BM-MSCs, the AT-MSCs also have tumor-homing potential. We have also optimized several *in vitro* strategies for isolation of cells with enhanced tumor-homing potential and increased invasion and migration towards tumor-derived factors. We have identified several surface markers that may enable enrichment of cells with the invasive phenotype. We have optimized the lentiviral transduction protocol in AT-MSCs, and have generated several stocks of cells stably expressing GFP. We have obtained the animal use approval and in the near future, we hope to show the enhanced tumor-homing of the enriched AT-MSCs to foci *in vivo*. This would facilitate the development of an optimal anti-cancer gene delivery vehicle to bone metastasized PCa.

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Appendices:

1. Poster Presentation at Tulane Health Science Research Day, March 3-4, 2010, New Orleans, LA.

ADIPOSE TISSUE DERIVED MESENCHYMAL STEM CELLS: INVASION, DIFFERENTIATION AND APPLICATION IN TARGETED DELIVERY OF DRUGS TO BONE METASTASIZED PROSTATE CANCER.

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Background: Human bone marrow derived multipotent mesenchymal stem cells (BM-MSCs) are a promising autologous source for producing biological agents locally at tumor sites. However, their therapeutic use has been limited essentially because collection of bone marrow is a painful procedure. Moreover, there is always a potential risk that the patient's cancer cells may already be in circulation in the bone marrow. Human adipose derived MSCs (AT-MSCs) on the other hand are easy to procure from liposuction aspirates and are capable of differentiating along multiple lineages at least *in vitro*. Thus a thorough understanding of their biology can be applied towards the ultimate goal of using these cells for various forms of therapy and tissue engineering. However, use of these cells particularly as anti-cancer gene delivery vehicles requires the knowledge of factors that regulate their invasive behavior and differentiation potential towards the desired cell fate. Accordingly, present study first compared to see if invasive and differential potential of AT-MSCs is comparable to that of currently used BM-MSCs to establish their suitability as delivery vehicle for treating bone metastasized prostate cancer.
Methods: Our initial studies were geared to assess the importance of growth factors and tumor microenvironment specific factors in the recruitment of AT-MSCs to tumors using *in vitro* invasion of MSCs through Matrigel-coated inserts. To further evaluate their differentiation potential towards osteogenic and adipogenic lineages, differentiation of AT-MMSC was assessed in response to prostate cancer (PCa) cell line specific conditioned media (CM) and quantification of differentiation by colorimetry following either Oil red-O or Alizarin red staining.
Results: Here we report that AT-MSCs were able to traverse through the matrigel inserts (percent invasion 98% - 104% for different donors) as well as the BM-MSCs (percent invasion 99% - 105%). In addition, serum deprivation and CM from PCa cell lines particularly the C4-2B and PC3 CM augmented (2.2 fold & 1.7 fold, respectively) their invasive behaviors. Studies to assess their differentiation potential demonstrated a trend towards adipogenic and osteogenic differentiation in the presence of tumor cell CM. Detailed temporal analyses of the invasive and differentiation programs are in progress. Finally in order for us to enrich for the invasive population of AT-MSCs, influence of bone marrow endothelial barrier on the invasive behavior is also being evaluated.
Conclusions: Our preliminary findings are relevant for devising enrichment strategies for tumor tropic AT-MSCs. Understanding how pathways mediating the differentiation between osteoblasts and adipocytes are regulated should be of relevance for the development of tumor tropic AT-MSCs that can be utilized for the therapeutic control of anti-cancer genes following their recruitment to bone metastasized prostate cancers.

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2. <u>Poster Presentation at the AACR Conference, April 17-21, 2010. Washington DC.</u>

Nrf1 and Nrf2 mediated regulation of androgen receptor signaling in androgen-independent prostate cancer cells. Michelle Schultz*, Asim B. Abdel-Mageed, Krishna C. Agrawal, Geetika Chakravarity and Debasis Mondal. Tulane University Medical Center, New Orleans, LA-70112.

Androgen deprivation is often used for the treatment of prostate cancer (PCa) patients. However, the development of androgen independent (AI) PCa remains a significant barrier to successful therapy. Interestingly, the androgen receptor (AR) is functionally expressed in all stages of PCa, although AI-PCa cells often overexpress AR and employ alternative signaling cascades to enhance AR signaling. Elevated reactive oxygen species (ROS) levels are associated with increased aggressiveness in PCa. Delineation of how these pathways are utilized by AI-PCa cells will implicate novel targets for abrogation of aggressive tumor cell growth in the later stages of PCa. We have used two sets of syngeneic PCa cell lines to emulate the progression of PCa *in vitro* from a non-tumorigenic stage (RWPE-1 and RWPE2 cells) to a tumorigenic and androgen-dependent (LNCaP cells) and androgen-independent (C4-2B cells) state. PSA luciferase assays showed that C4-2B cells have 7-8 fold higher activation of the AR regulated gene (PSA) than LNCaP cells after 1nM and 10nM DHT treatment for 24hrs. We have also observed that LNCaP and C4-2B cells have the ability to produce higher levels of (ROS), most likely due to an increased expression of the ROS producing enzymes, the NADPH oxidases, NOX4 and NOX5. Maintenance of higher ROS levels in C4-2B cells may allow red-ox pathways to be used for mitogenic purposes. These cells also overexpress the antioxidant proteins, Thioredoxin-1 (Trx-1) and Peroxiredoxin-1 (Prx-1), which may prevent the detrimental effects of long term exposure to ROS produced by NOX4 and NOX5. Red-ox pathways have been shown to modify hormone receptor signaling in both breast and prostate cancer. Trx-1 can differentially modulate estrogen receptor (ER) signaling in breast cancer and Prx-1 is known to bind directly to AR and enhance AR transactivation in PCa cells. The overexpression of these antioxidant proteins in AI-PCa suggests that these cells can use antioxidants to enhance AR mediated signaling, even under androgen deprived conditions. Hence, suppression of AR dependent PCa cell growth may be achieved by suppressing the induction of Prx-1 and Trx-1. Induction of these antioxidant proteins are dictated by two redox sensitive transcription factors, Nrf1 and Nrf2. C4-2B cells showed the highest expression of Nrf1 and the lowest expression of Nrf2 at the mRNA and protein levels. Indeed, decreased Nrf2 expression has also been associated with PCa aggressiveness. It is likely that a balance between Nrf1 and Nrf2 signaling regulates the expression of Prx-1 and Trx-1, which may ultimately regulate AR activity in C4-2B cells. A clearer understanding of how the Nrf1/Nrf2 balance mediates antioxidant protein expression and AR mediated gene regulation may lead to new avenues for treatment of hormone independent PCa cells, via gene-therapy approaches targeting the Nrf1/Nrf2 balance in tumor-microenvironments.

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